SDA NONIONIC METHOD 583G

Determination of Ethoxylated Alcohol Surfactants in Wastewater by Liquid Chromatography

SCOPE

This method is a series of procedures designed to isolate,purify, and determine the quantity of ethoxylated alcohol surfactants in wastewater streams. The method is selective for alcohol ethoxylates, with a detection limit of approximately 0.1 ppm

KEYWORDS

Nonionic surfactants
Surfactants
Wastewater
Liquid chromatography
Soap and Detergent Association
Alcohol ethoxylate
AE
Cobalt thiocyanate active substance
CTAS

PRINCIPLE

The nonionic surfactant in the sample is isolated from its matrix by chromatography on XAD-2 resin, sublation (or extraction), ion-exchange, and alumina column chromatography. The purified residue from these procedures is then analyzed by CTAS and by high performance liquid chromatography (HPLC).

The method is outlined in Figure 1.

APPARATUS

1. Flowmeters capable of measuring nitrogen flow rates in the range 20 to 60 liters per hour (e.g., Air Products and Chemicals Catalog No. E29-4-150MM3).
2. Nitrogen gas supply capable of delivering a nitrogen flow rate in the range 20 to 60 liters per hour.
3. Sublators (see Figure 2). An equivalent apparatus is available by special order from SGA Scientific Inc., Bloomfield, New Jersey.
4. Chromatography column, glass, for XAD-2 resin. See Figure 3.
5. Reservoir, 500 mL, made to fit XAD-2 column.
6. Chromatography columns, glass, for ion exchange cleanup. See Figure 4A.
7. Chromatography columns, glass, for alumina cleanup. See Figure 4B.
8. Separatory funnels with Teflon stopcocks, 125 and 250 mL capacities.
9. Pasteur pipets with 2 mL latex bulbs.
10. Filter paper, Whatman No. 1, 15 cm diameter.

    NOTE 1: It is advisable to Soxhlet extract the filter paper overnight with methanol before using for samples containing very low levels of nonionic surfactants.
11. Stirring rods, glass, 3 mm x 12 cm.

12. Centrifuge capable of 3,500-4,000 rpm, Damon/IEC Clinical Model or equivalent.
13. Centrifuge tubes, glass, 10 or 15 mL, screw capped.

14. Steam bath.
15. Spectrophotometer, Spectronic 70 or equivalent, equipped with Teflon-stoppered cells of 2 cm pathlength.
16. Heat lamp.
17. High quality liquid chromatograph with binary solvent programming capability. It must be equipped with an UV absorbance detector which can operate at 235 or 240 nm. An electronic integrator is desirable.
18. Reverse phase LC column, Waters μBondapak C18, 3.9 x 300 mm.
19. Normal phase LC column, Waters μBondapak NH_2, 3.9 x 300 mm.
20. Apparatus to filter the LC solvents using 0.45 micron membrane filters. Cellulose acetate membrane filters such as Gelman type GA-6 or Millipore type HA are suitable for aqueous solvents. A Teflon filter such as Millipore type FH should be used for organic solvents.
21. Vacuum oven.
22. Extraction flask, 250 mL.
23. Extraction flask, 500 mL.
24. Vials, glass, 2 dram and 1 dram
25. Desiccator.
26. Analytical balance and ordinary laboratory glassware.

REAGENTS

1. Deionized water, HPLC grade, purified with a Millipore Milli-Q filtration system or equivalent.
2. Sodium chloride, reagent grade.
3. Sodium bicarbonate, reagent grade.
4. Sodium sulfate, anhydrous, crystalline, reagent grade.
5. Formaldehyde (37 percent aqueous solution), reagent grade.

6. Ethyl acetate, Burdick and Jackson distilled-in-glass, or equivalent.
7. Methanol, Burdick and Jackson distilled-in-glass, or equivalent.
8. Hexane, Burdick and Jackson distilled-in-glass, or equivalent.
9. Ethanol, SD Formula 30, 190 proof. Prepare by mixing 950 mL absolute ethanol with 50 mL deionized H_2O. To each 100 mL of this mixture, add 10 mL reagent grade methanol.
10. Methylene chloride, Burdick and Jackson distilled-in-glass, or equivalent.
11. Cobalt thiocyanate solution, dissolve 15 g $Co(NO_3)_2 \cdot 6H_2O$ (reagent grade) and 100 g NH_4SCN (reagent grade) in deionized water and dilute to 500 mL. The solution is stable for at least one month at room temperature.
12. Nonionic surfactant reference material: a 50:50 blend of NEODOL® 25-9 and ALFONIC® 1218-70.
13. Alumina, neutral, Brockman Activity No. 1, 80-200 mesh, Fisher Scientific Company Catalog No. A950.
14. Deactivated alumina. The water content of the alumina must be controlled, since it will affect the retention volume. The Fisher neutral alumina used contains approximately 3 percent water. The water content is increased to 5 percent according to the following procedure:
    a. Determine the water content of the "as received" alumina by calcining approximately 5 g (accurately weighed) in a 50 mL tared platinum dish for one hour at 900°C. Cool the sample in a desiccator before reweighing. Calculate the weight loss as weight percent water.
    b. Adjust the water content of the original "as received" alumina by adding the amount of water necessary to make 100 grams of deactivated alumina, e.g., if the "as received" alumina contains 3% H_2O, use 2 g of water and 98 g of the "as received" alumina. The water should be added to a clean, dry, amber glass bottle. Swirl and turn the bottle to wet the sides and bottom. Add enough "as received" alumina to make 100 g of 5 weight percent material. Roll and tumble the bottle to mix the contents well. Allow it to equilibrate overnight
    c. Before using, confirm that the water content is indeed 5% by repeating step a.

15. Anion exchange resin, Bio-Rad AG1-X2, chloride form, 50-100 mesh.
16. Cation exchange resin, Bio-Rad AG50W-X8 acid form, 50-100 mesh.
17. Isopropanol, Burdick and Jackson, distilled-in-glass or equivalent.
18. Sodium chloride solution, 5 M. 292 g reagent grade NaCl per liter. Prepare with deionized water.
19. Phenyl isocyanate, reagent grade.
20. Hydrochloric acid, concentrated reagent grade.
21. Amberlite XAD-2 resin, purified, Applied Science Division, Milton Roy Company Laboratory Group. Catalog No. 17311.
22. Sodium hydroxide solution, 0.05 M in methanol. Dissolve 1.0 g reagent grade NaOH in 500 mL reagent grade methanol. Standardization is not necessary.
23. Chloroform with hydrocarbon stabilizer but without ethanol stabilizer, Burdick and Jackson distilled-in-glass, or equivalent.
24. Petroleum ether, Burdick and Jackson distilled-in-glass, or equivalent.
25. Ethylene dichloride, Burdick and Jackson distilled-in-glass, or equivalent.
26. Tetrahydrofuran, Burdick and Jackson distilled-in-glass, or equivalent.
27. Ethyl ether, Burdick and Jackson distilled-in-glass, or equivalent.
28. Acetone, Burdick and Jackson distilled-in-glass, or equivalent.
29. Acetonitrile, Burdick and Jackson distilled-in-glass, or equivalent.
30. Hydrochloric acid/chloroform/methanol mixture. Add 5 mL concentrated HCl to a mixture of 25 mL $CHCl_3$ and 25 mL methanol.
31. Internal standard solution. Weigh 0.250 g 1-octanol and 0.25 g 1-eicosanol into a 100 mL volumetric flask. Dissolve and dilute to volume with ethylene dichloride. Stopper tightly. This solution contains 2.5 mg/mL each of C_8 and C_{20} primary alcohols.

32. Sodium hydroxide solution, 1.0 M. Dissolve 40 g reagent grade NaOH in 1 L deionized water. Standardization not necessary.
33. Hydrochloric acid, 0.1 M. Dilute 8.3 mL concentrated HCl to 1 L with deionized water. Standardization not necessary.
34. Phenolphthalein indicator solution. 0.1% in ethanol.
35. Reference standard solution, 1 mg/mL. Dissolve 0.100 g of the 50:50 alcohol ethoxylate blend in methylene chloride in a 100 mL volumetric flask. Make up to volume with methylene chloride.

SAFETY CONSIDERATIONS

Some corporate medical departments require special precautions for personnel working with sewage samples, e.g., inoculation against typhoid fever.

Ethyl acetate, acetonitrile, methanol, ethanol, isopropanol, ethylene dichloride, and especially THF, acetone, ethyl ether, hexane and petroleum ether are flammable solvents.

Ethyl acetate has a strong odor and is a mild irritant. Methanol, ethyl ether and chloroform have a narcotic effect. Phenyl isocyanate can cause an allergic reaction to a sensitized individual. Ethylene dichloride, chloroform, and formaldehyde are suspected carcinogens. All of these should be used in the hood. Vapors are to be kept out of the laboratory air.

PROCEDURE

Part I - Concentration of Surfactants from the Sample with XAD-2 Resin

1. Place a plug of glass wool near the bottom of the chromatographic column. Fill the column to a depth of approximately 7 inches with a methyl alcohol slurry of the XAD-2 resin, and place a plug of glass wool tightly against the top of the resin bed. Prepare a new column by following the procedure in the Note, below.
2. Select a sample volume calculated to contain at least 1.0 mg nonionic surfactant and pass through the column at a rate of approximately 1 drop/sec. Sample size should be about 500 mL for an influent sample and about 10 L for an effluent sample.

3. Rinse resin with 30 mL petroleum ether. Apply pressure with a suction bulb to force the petroleum ether through the resin. Force all of the petroleum ether out of column. Discard.

4. Place a 250 mL extraction flask under the column and elute sequentially with 40 mL ethyl ether, 60 mL 1:1 ethyl ether/-methanol, and 30 mL methanol. Maintain a flow rate of 1 drop/second. If necessary, use a rubber bulb to force the ether through the column. (See note.)
5. Evaporate eluate from (4) to approximately 5 mL on steam bath under a stream of nitrogen. Add 5 mL water and continue evaporation to approximately 5 mL.
6. Proceed with Part II, Sublation of Samples, or Part IIa, Liquid-Liquid Extraction.

NOTE:

The packed XAD-2 column can be used indefinitely if the following regeneration sequence as followed:

a. After completing Part I through Step 4, above, rinse the column sequentially with 40 mL 0.05 M NaOH in methanol, 55 mL HCl/methanol/chloroform, and 25 mL methanol. Discard.
b. Replace the upper glass wool plug.
c. Attach to the delivery tip of the column a suction line connected to a water aspirator.
d. Invert the column, dip the top in a beaker of deionized water, and draw about 250 mL water through the resin.
e. Close the stopcock and rapidly turn the column right-side-up. Disconnect the suction line.

Part II - Isolation of Surfactants by Sublation (Used for samples which tend to form emulsions during conventional liquid-liquid extraction)

1. Rinse the concentrated XAD-2 eluate from Part I into a 1 L beaker with 600-700 mL deionized water. While stirring, add 100 g NaCl and 5 g $NaHCO_3$. Continue stirring until dissolved. Add more water, rinsing the walls of the beaker, to make a final volume of 800 mL.

2. Quantitatively transfer the above solution to the sublator. Rinse the beaker a few times with deionized water, and transfer the washings to the sublator. Add enough water to bring the liquid level to the level of the upper stopcock.
3. Add 100 mL ethyl acetate to the sublator by pipet so as to avoid mixing with the aqueous solution.
4. Turn on the nitrogen, and pass it through the sublator at the fastest rate possible, while avoiding mixing at the ethyl acetate/water interface.
5. Sublate until 4 L of nitrogen have passed through the solution, e.g., 40 L per hour for 6 min.
6. Stop the nitrogen flow, allow the phases to separate, and drain the ethyl acetate layer into a 500 mL separatory funnel. Return any aqueous phase to the sublator.
7. Repeat Steps 3 through 6 until a total of four 100 mL portions of ethyl acetate have been collected in the separatory funnel.
8. Rinse the sublator with two 25 mL portions of ethyl acetate. Add the washings to the separatory funnel. Discard any aqueous phase in the separatory funnel.
9. Add 10-15 g anhydrous sodium sulfate to the separatory funnel, stopper, and invert 4-5 times. Allow to stand a few min, then filter through Whatman No. 1 filter paper containing an additional 10-15 g sodium sulfate. Collect the filtrate in a 500 mL extraction flask.
10. Rinse the separatory funnel with two 25 mL portions of ethyl acetate. Filter the washings into the extraction flask.
11. Finally, rinse the filter paper containing the sodium sulfate with two 25 mL portions of ethyl acetate.
12. Evaporate the ethyl acetate in the extraction flask on a steam bath with the aid of a gentle stream of dry nitrogen, just to dryness. Remove from the heat immediately.
13. Dissolve the residue in 10 mL methanol and save for ion exchange chromatography (Part III).

Part IIa - Isolation of Surfactants by Liquid-Liquid Extraction)

1. Rinse the solution from Part I (5) with 45 mL 5M NaCl solution into a 250 mL separatory funnel containing 50 mL ethyl acetate.

2. Shake the funnel vigorously for 1 min and allow the layers to separate.

3. Drain the lower, aqueous layer into a second separatory funnel and add 50 mL of 5M NaCl solution to the first funnel. Shake funnel for 1 min and allow layers to separate.

4. Again drain the lower layer into the second funnel. Swirl the contents of first funnel and drain separated aqueous layer (1-2 mL) into second funnel.

5. Add 50 mL ethyl acetate to second funnel and shake for 1 min.

6. Allow layers to separate and then drain and discard the lower aqueous layer.

7. Decant the ethyl acetate layer remaining into the first separatory funnel, add two heaping teaspoons of anhydrous sodium sulfate, and shake for 10-15 sec.

8. Filter the ethyl acetate extract through folded 9 cm Whatman No. 40 filter paper containing 2 heaping teaspoons of

anhydrous sodium sulfate, collecting filtrate in a 150 mL extraction flask.

9. Rinse the second separatory funnel with 25 mL ethyl acetate and decant into the first separatory funnel. Again shake 10-15 sec and filter through the Na_2SO_4.

10. Repeat step 9.

11. Evaporate the ethyl acetate in the extraction flask on a steam bath with the aid of a gentle stream of dry nitrogen, just to dryness. Remove from the steam bath immediately.

12. Dissolve the residue in 10 mL methanol and save for ion exchange chromatography (Part III).

Part III - Ion Exchange Separation of Nonionic Surfactants

A. Preparation of Ion Exchange Columns

1. Place a glass wool plug in the bottom of an ion exchange column, just above the stopcock.
2. Slurry about 20 g of the anion exchange resin in water, and transfer to the ion exchange column.
3. Elute with 50 mL of 1M aqueous sodium hydroxide at a rate of 1 to 2 drops per second.
4. Wash with water until one drop of 0.1M hydrochloric acid is sufficient to discharge the phenolphthalein color in 50 mL of effluent.
5. Elute with 50 mL of methanol to displace the water. Air pockets and channels must be removed before use. This can be done by inserting a serum cap in the tip of the ion exchange column and forcing methanol back up through the column using a 50 mL hypodermic syringe.
6. Place a glass wool plug on top of the anion resin bed.
7. Slurry approximately 16 g of cation exchange resin in methanol, and add to the ion exchange column.
8. Rinse the column with 50 mL of methanol. The column is ready for use in Part IIIB.

   NOTE: The ion exchange column may be used for up to six samples before repacking.

B. - Removal of Interfering Ionic Surfactants by Ion Exchange Chromatography

1. Quantitatively transfer the methanol solution of the residue from Part II or IIa to the ion exchange column using two 10 mL methanol rinses. Allow the methanol level to fall just to the top of the resin bed before adding each successive rinse. Collect the effluent in a 150 mL beaker. Continue eluting with methanol at an elution rate of 1-2 mL/min until a total of 125 mL of effluent has been collected.
2. Evaporate the methanol just to dryness on a steam bath with the aid of a gentle stream of nitrogen.

NOTE: Do not evaporate until ready to run CTAS analysis.

3. Proceed to Part IV for CTAS analysis.

Part IV - CTAS Analysis

A. Preparation of Calibration Curve

1. Pipet 1.00, 2.00, 3.00, and 4.00 mL of the reference standard solution into a series of 4 150 mL beakers. Evaporate just to dryness. These beakers contain 1, 2, 3, and 4 mg surfactant, respectively.
2. Pipet 10.0 mL methylene chloride into each of the beakers containing the evaporated standards. Stir for a few sec, and then quickly transfer each to a 125 mL separatory funnel. Stopper the funnel.
3. Pipet 5.0 mL of cobalt thiocyanate solution into the separatory funnel, stopper, and shake vigorously for 60 sec.
4. After the phases have separated, drain the lower, methylene chloride layer into a centrifuge tube. Cap and centrifuge at approximately 4,000 rpm for three min.
5. Using a Pasteur pipet, transfer the centrifugate to a 2 cm pathlength cell, stopper, and measure the absorbance at 620 nm versus CH_2Cl_2.

   NOTE: Rarely, a haze will develop in the sample cell because of cooling of the water-saturated methylene chloride solution. The stoppered cell may be warmed in the hand or with a heat lamp to remove this turbidity and thereby avoid a substantial error in the absorbance reading.
6. For samples, retain all the solutions, the beaker, the separatory funnel, the centrifuge tube, the transfer pipet, etc., for Part IV C.
7. Obtain the net absorbance by subtracting the absorbance of the reagent blank from that of the standard.

8. Prepare a calibration curve by plotting net absorbance versus milligrams of ethoxylate (CTAS). The slope should lie in the range of 0.1 to 0.3 absorbance units per milligram CTAS.

B. Analysis of Sample.

1. Pipet 10.0 mL methylene chloride into the beaker containing the evaporated sublated sample from Part III. Stir for a few sec, then quickly transfer to a 125 mL separatory funnel. Insert stopper.
2. Follow steps 3-7 of Part IV A.
3. The number of milligrams of CTAS in the final residue is determined by comparing its net absorbance to the standard curve. The concentration of CTAS in the original sample is calculated as follows:

$$\text{CTAS, ppm} = \frac{\text{mg from calibration curve}}{\text{Liters of sample used}}$$

C. - Recovery of Ethoxylates from CTAS Solution

1. Obtain, for a particular sample or standard, the solutions, the beaker, the separatory funnel, the centrifuge tube, the transfer pipet, etc. from the CTAS analysis.
2. After measuring the absorbance, transfer all the methylene chloride-CTAS solution to the retained beaker. Extract the aqueous CTAS solution in the separatory funnel with two additional 10 mL portions of methylene chloride and add the extracts to the beaker. Rinse the centrifuge tube, transfer pipet, cuvettes, etc., used in the CTAS analysis with an additional 10 mL of methylene chloride and add the rinsings to the beaker.
3. Quantitatively transfer the methylene chloride-CTAS solution from the beaker to a clean 250 mL separatory funnel using two 10 mL portions of methylene chloride to rinse the beaker.
4. Add 50 mL of 5M aqueous sodium chloride solution, shake well, and allow the phases to separate.

5. Pass the methylene chloride phase (lower layer) through 10-15 g anhydrous sodium sulfate (in a cone of Whatman No. 1 filter paper held in a funnel) into the 150 mL beaker.
6. Extract the salt solution with two additional 10 mL portions of methylene chloride. Dry the extracts by passing them through the sodium sulfate.
7. Wash the sodium sulfate and filter paper with 20 mL methylene chloride.
8. Evaporate all the extracts and washings on a steam bath using a stream of nitrogen to hasten evaporation.
9. Proceed with the alumina column purification in Part V.

Part V - Alumina Column Chromatographic Purification of Samples for HPLC (Optional)

1. Insert a glass wool plug into the alumina column.
2. Slurry 10 g of deactivated alumina (5 percent H_2O) in hexane and transfer to the column.
3. Wash the column with 50 mL hexane.
4. Transfer the residue from Part IV to the alumina column using several 10 mL hexane rinses. Elute at a rate of one to two drops per second. Allow the hexane level to fall just to the top of the alumina bed before adding each successive rinse.
5. Continue elution with hexane until a total of 100 to 125 mL of hexane has passed through the column. Discard the hexane.
6. Rinse the sample beaker several times with 10 mL portions of Formula 30 (no substitutions!) denatured ethanol. Add the ethanol rinses to the column. Collect the effluent in a 150 mL beaker. Allow the ethanol level to fall just to the top of the alumina bed before adding each successive rinse.
7. Continue elution with Formula 30 ethanol until 100 mL of effluent has been collected.
8. Evaporate the ethanol just to dryness on a steam bath with the aid of a gentle stream of nitrogen.
9. Transfer the residue in the beaker from the alumina column into a 2 dram glass vial with several rinses of methylene chloride. Evaporate to dryness with the aid of a gentle stream of nitrogen.

Part VI - Derivatization with Phenyl Isocyanate

1. Prepare an EDC solution containing 2.5 mg/mL of each of the C_8-C_{20} primary alcohols. Transfer 50 µL of this standard solution to a 1 dram vial. Add 10 µL phenyl isocyanate.
2. To the vial containing the residue from Part V, add 50 µL alcohol internal standard solution (Reagent #31) and 10 µL phenyl isocyanate.
3. Rinse the walls of the vials with 50 µL ethylene dichloride. Swirl to mix, cap loosely, and allow to react 45 minutes at 55 ± 2°C in a vacuum oven under 20-30 inches vacuum.
4. Dissolve the residue in 250 µL ethyene dichloride.

PART VII - HPLC Separation - Reverse Phase

1. LC conditions for separation by alkyl chain length:

   Column: µBondapak C18 (Waters Associates) 3.9 x 300 mm
   Detector: UV Absorbance 1 AUFS, 235 or 240 nm

   Solvent A: Water
   Solvent B: Methanol
   Gradient: 80% B to 100% B, linear in 30 min.
   Flow rate: 2.0 mL/min
   Injection volume: 10 µL for influent samples
   20 µL for effluent samples

2. Analyze the sample and standard according to the above conditions. See Figure 5 for a typical chromatogram. The chromatogram of the standard is used for peak identification. The peak areas from the sample chromatogram are used in the calculations.

Part VIII - HPLC Separation - Normal Phase (Optional)

1. LC conditions for separation be degree of ethoxylation:

   Column: μBondapak NH_2 (Waters Associates), 3.9 x 300 mm
   Solvent A: Hexane/ethylene dichloride, 350/150
   Solvent B: Acetonitrile/isopropanol, 185/65 (add 200 μL acetone)
   Gradient: 0% B to 35% B, linear in 50 min.
   Flow rate: 3.0 mL/min
   Detector: UV Absorbance 0.1 AUFS, 235 or 240 nm
   Injection volume: 10 μL for influent samples
   20 μL for effluent samples

2. Analyze another portion of the derivatized sample from Part VI by the above conditions.

NOTE: The mobile phase and solvent gradient may have to be modified somewhat to compensate for the characteristics of different columns.

Part IX - Calculations

1. The peak areas from the first chromatogram (by alkyl chain) are used to calculate the micrograms of AE in the sample as shown in Example A, assuming an average degree of ethoxylation of 9 (FW = 396) for influent samples and 3 (FW = 132) for effluents.
2. Optionally, the peak areas from the second chromatogram (by ethoxyl number) are used to calculate the average molecular weight of the ethoxyl chain as shown in Example B. This value is then substituted for 396 in the calculation for Example A.
3. The total micrograms of AE in the sample, and ppm AE in the sample, are then calculated as shown in Example C.

EXAMPLE A

Alkyl Chain	Area of Alkyl Peak / Area of C_8 Alc Peak*		MW of Alc + MW of Ethoxy / MW of C_8 Alc*		Total µg C_8 or C_{20} Alcohol		µg AE Alkyl Chain
11	$\frac{7.1062}{20.4652}$	x	$\frac{172 + 396}{130}$	x	125	=	189.6
12	$\frac{5.9270}{20.4652}$	x	$\frac{186 + 396}{130}$	x	125	=	162.1
13	$\frac{7.8265}{20.4652}$	x	$\frac{200 + 396}{130}$	x	125	=	219.2
14	$\frac{5.4966}{20.4652}$	x	$\frac{214 + 396}{130}$	x	125	=	157.5
15	$\frac{1.6134}{20.4652}$	x	$\frac{228 + 396}{130}$	x	125	=	47.3
16	$\frac{.6550}{20.4652}$	x	$\frac{242 + 396}{130}$	x	125	=	19.6
18	$\frac{.7776}{20.4652}$	x	$\frac{270 + 396}{130}$	x	125	=	24.3
					Total µg AE	=	819.6

* The C_{20} alcohol peak may be used for these calculations, using a MW of 298.

See Example A-2 for simplified calculations when degrees of ethoxylation are assumed.

EXAMPLE A-2

For Influent Samples (where an average degree of ethoxylation of 9 is assumed)

$$\frac{\text{Area of Alkyl Peak}}{\text{Area of } C_8 \text{ or } C_{20} \text{ Alcohol Peak}} \times \text{Factor} = \mu g \text{ AE for } R_n \text{ Alkyl Chain}$$

Alkyl Chain	Factor for C_8 Alcohol	Factor for C_{20} Alcohol
11	546.2	238.3
12	559.6	244.1
13	573.1	250.0
14	586.5	255.9
15	600.0	261.7
16	613.5	267.6
18	640.4	279.4

Total µg of EA = Σ of µg/alkyl chain

For Effluent Samples (where an average degree of ethoxylation of 3 is assumed)

Alkyl Chain	Factor for C_8 Alcohol	Factor for C_{20} Alcohol
11	292.3	127.5
12	305.8	133.4
13	319.2	139.3
14	332.7	145.1
15	346.2	151.0
16	359.6	156.9
18	386.5	168.6

EXAMPLE B

Molecular Weight Calculation for Ethoxylates

Ethoxyl Number	Area	Fractional Area	x	M.W.	=	Partial M.W.
1	1.6257	.005		44		0.2
2	7.0863	.023		88		2.0
3	12.5568	.041		132		5.4
4	20.8083	.068		176		12.0
etc.						etc.

307.5984 = Total area

391.8 = Average Ethoxyl MW

EXAMPLE C

$$\text{ppm AE} = \frac{\text{Total } \mu g \text{ of AE in Sample}}{\text{mL of sample (influent or effluent)}}$$

$$\text{ppm AE} = \frac{820\ \mu g}{250\ mL} = 3.28$$

FIGURE 1

OUTLINE OF METHOD

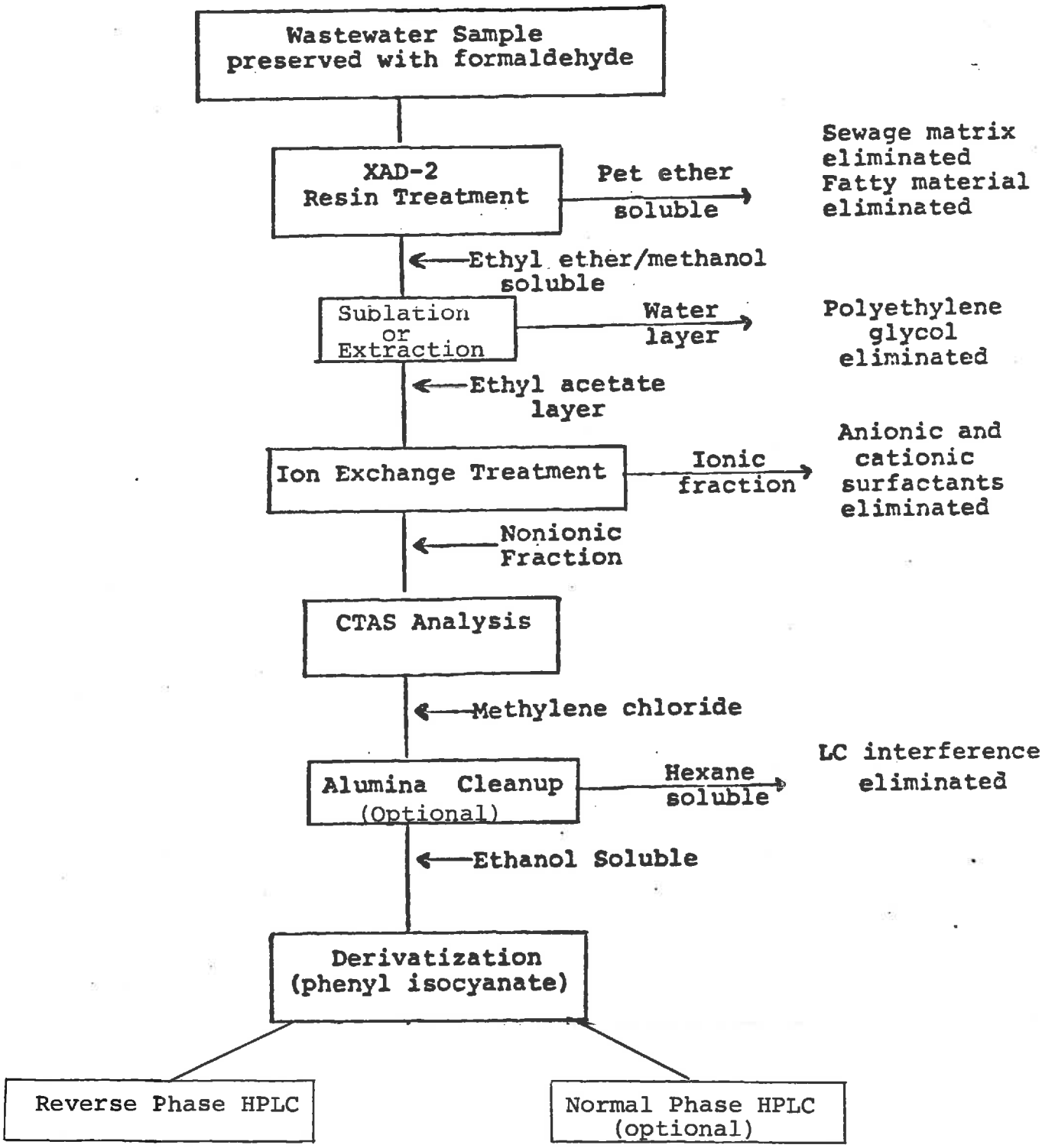
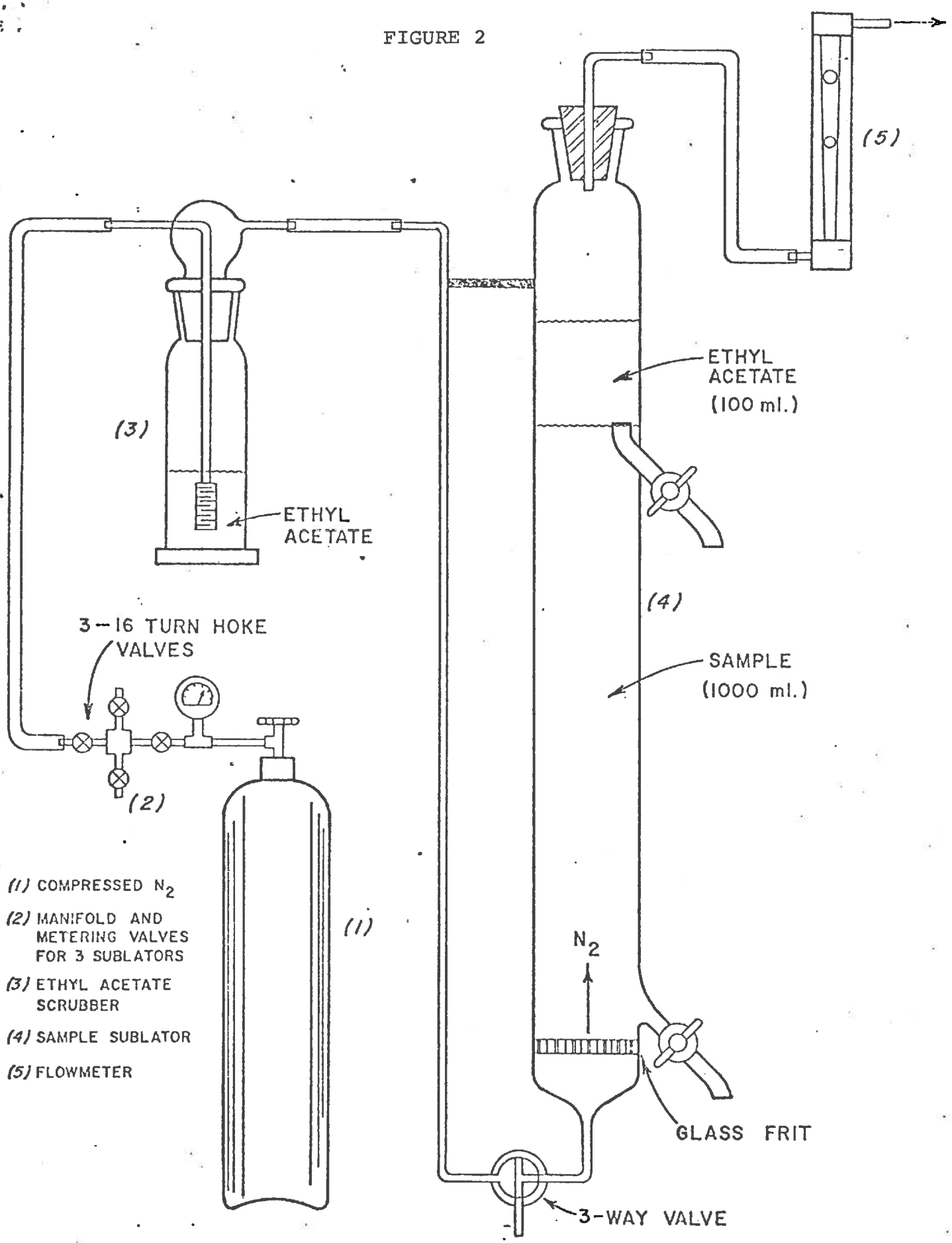
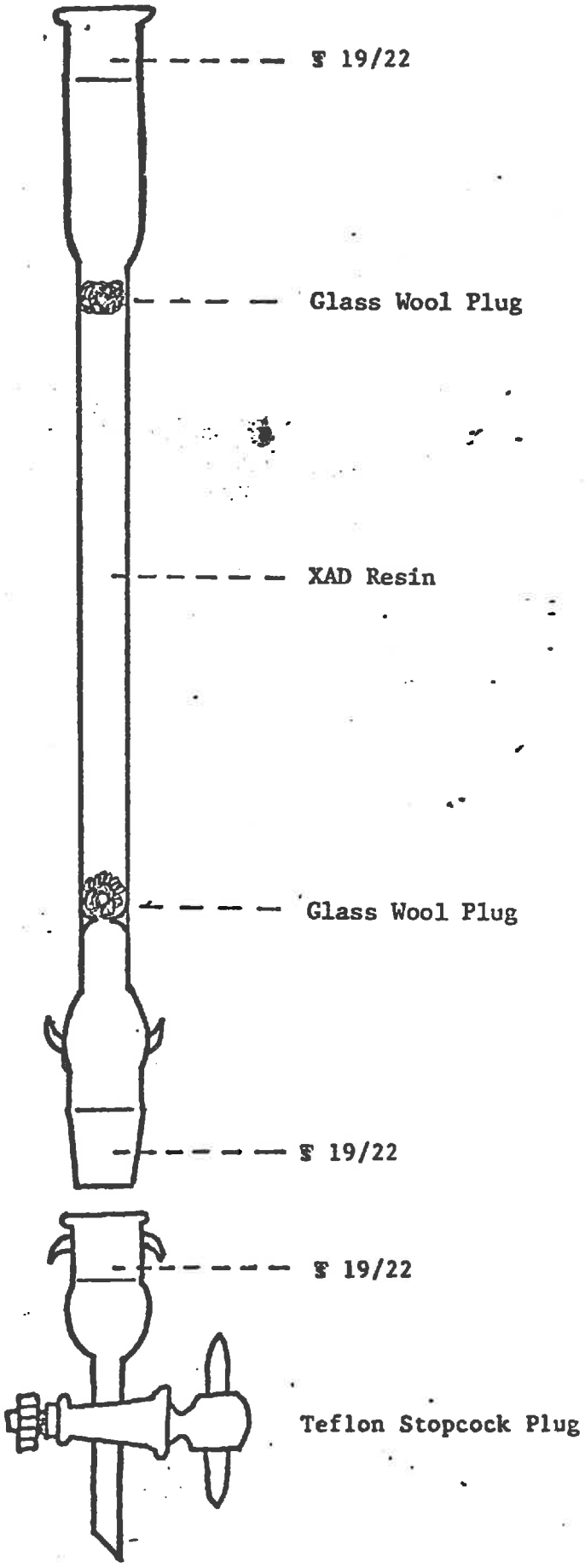


FIGURE 2



SUBLATION APPARATUS

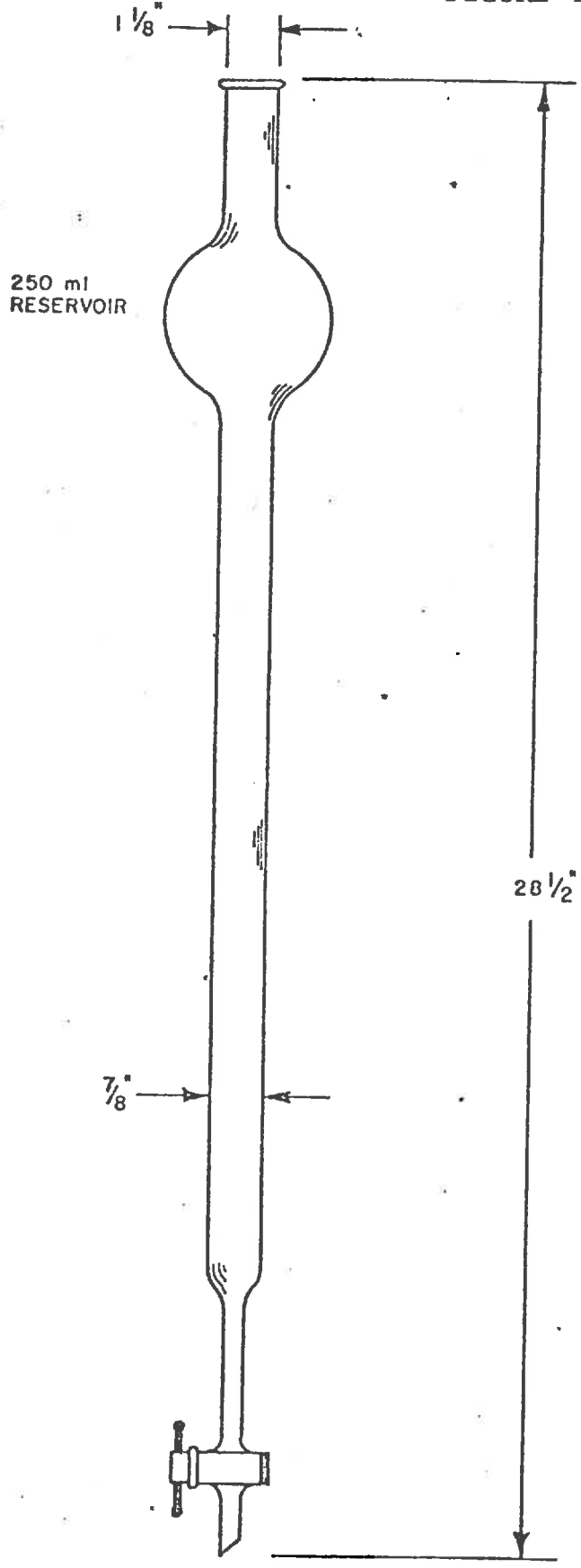
FIGURE 3



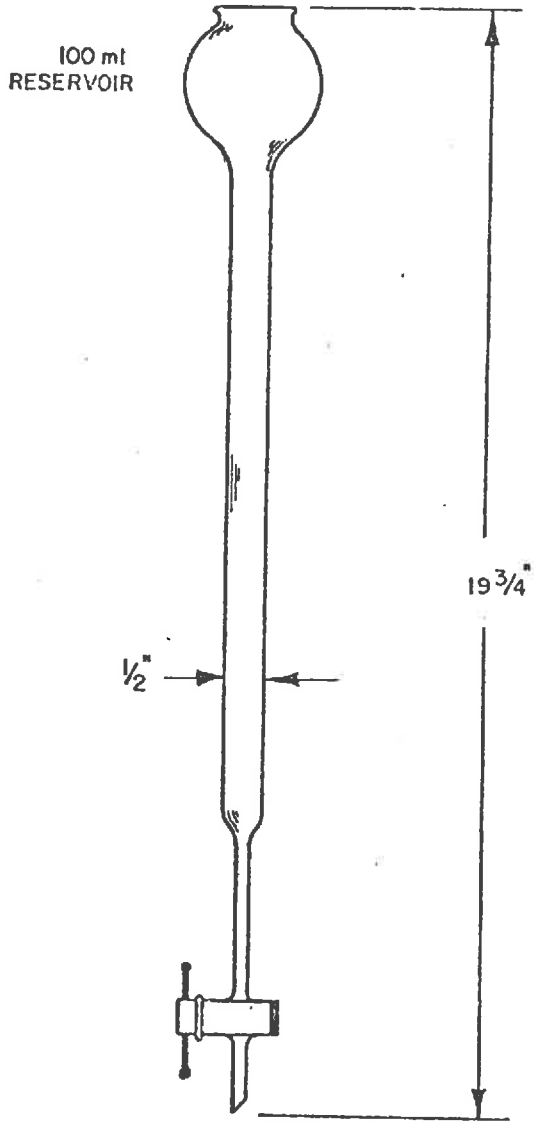
Scale=½ Actual Size

Chromatographic Column

FIGURE 4



ION EXCHANGE COLUMN



ALUMINA COLUMN

FIGURE 5

Reverse Phase HPLC Separation
Differentiation According to Alkyl Chain Length

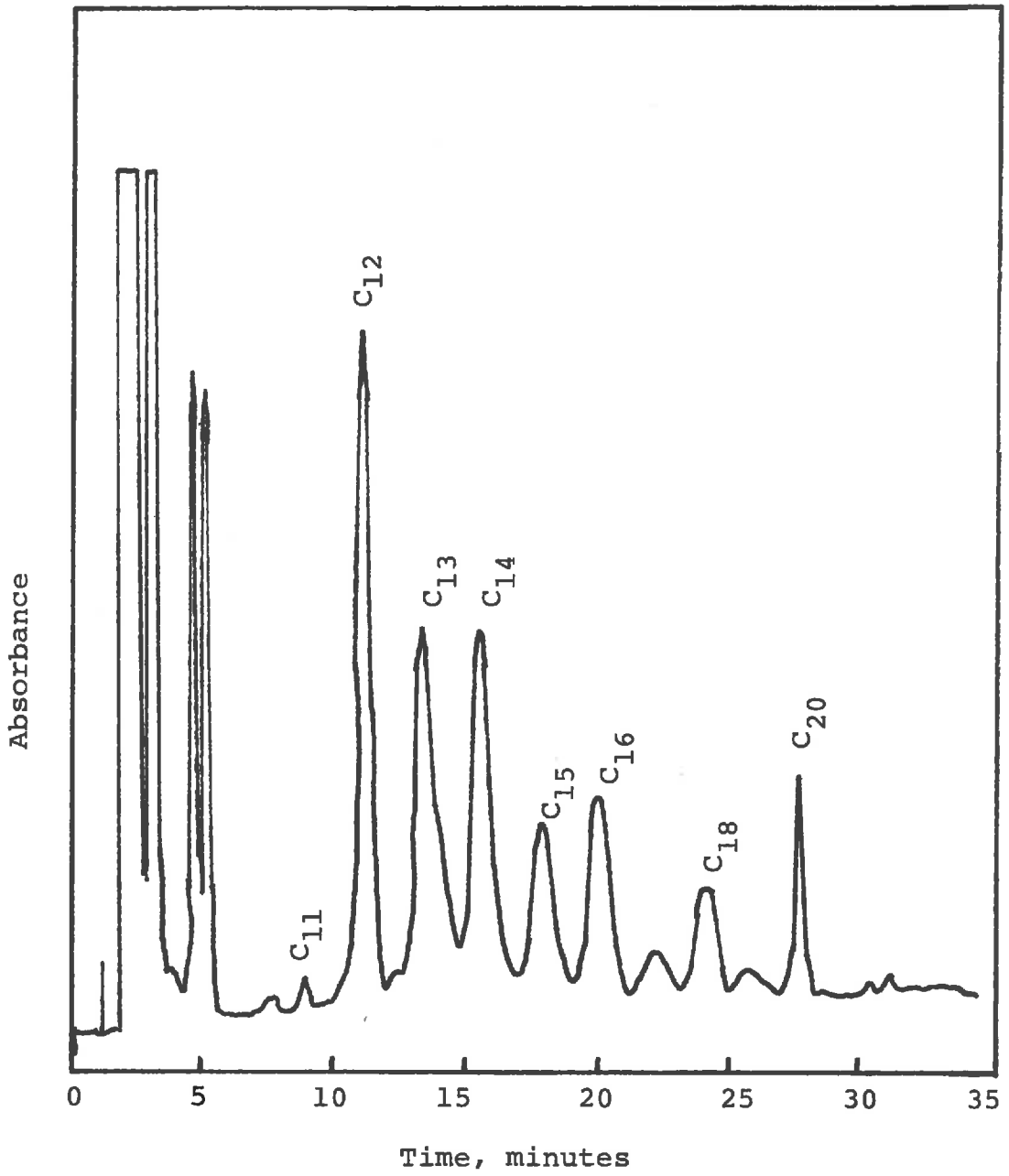


FIGURE 6

Normal Phase HPLC Separation
Differentiation According to Extent of Ethoxylation